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Air Quality Monitoring using a Whole-Cell based Sensor System

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Abstract

In this work, a novel whole-cell based biosensor for the detection of toxic substances in air was established. Due to their basic necessity of liquid environment, cell-based biosensors were underrepresented in the field of gas sensors in the last decades. The adaption of a commercial sensor chip (Bionas®) for the measurement of pollutants in liquids enables the direct exposure of cells with air. Cells of the respiration tract (A549, RPMI2650, V79), which tend to survive at a gas phase, are used as biological receptors. Three physiological cell parameters are monitored continuously in parallel (acidification, respiration, morphology). Water insoluble gases (e.g. CO) as well as water soluble gases (e.g. NH_3) were used as model gases to test the feasibility of the novel sensor system. MIR-measurements proofed the reproducibility of the draining method. This sensor system provides a basis for many sensing applications such as environmental monitoring, building technology and public security.

Keywords: cell-based gas biosensor, whole-cell based sensor system, NH_3, CO, A549, RPMI2650, V79, MIR

1. Introduction

In the last decades, air and gas-monitoring was mainly a topic for solid-state and optical sensors whereas gas biosensors were underrepresented [1]. These classical air monitoring technologies lack of the possibility to measure “toxicity” as a parameter itself. In contrast, living cells have the possibility to react towards a broad range of different pollutants [2].

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The aim of the work was to adapt a cell-based biosensor for the use in a gaseous environment to measure toxicity in air/gas-mixture [3]. As a transducer unit we used a commercial sensor chip (Fig. 1) which is capable of the measurement of three different physiological parameters (acidification, respiration and cell morphology). The sensor chip acts as a Petri dish where the cells are constantly fed with nutrition medium via a fully automated perfusion system. Up to date, this system was just used for the detection of toxic substances in liquid medium. Meanwhile, the system can be used in an adapted mode for the detection of toxicity in gas phases.

2. Experimental

2.1. Cell adhesion measurement

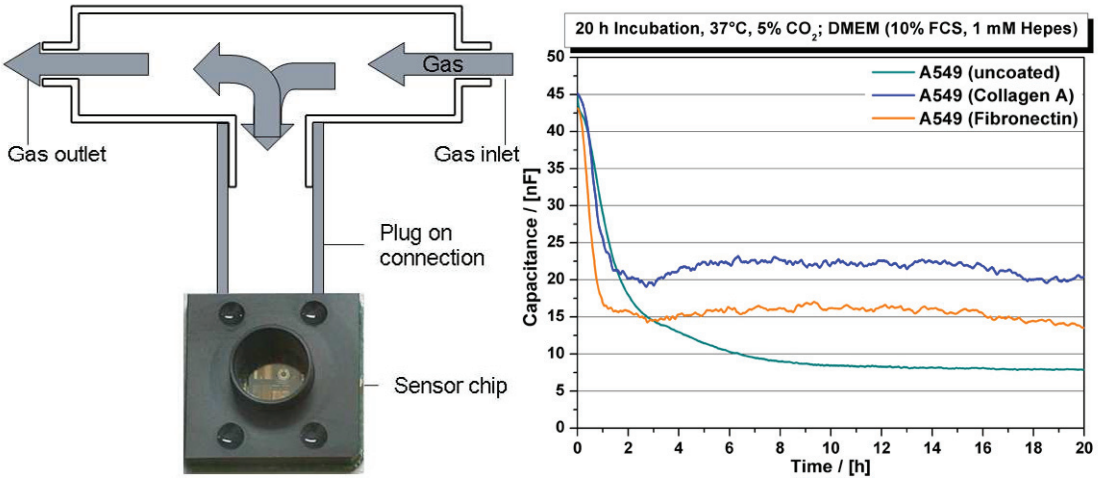
Cell sensor chips were used in an open which allows the direct access of gaseous substances. The whole sensor system is placed inside an incubation hood which is stable tempered at 37°C. Cells in a Hepes buffered 10% FCS running medium were transferred into the culture vessel of the cell sensor chip. The surface of the sensor chip was coated previously with different types of extra cellular matrix proteins (collagen A, fibronectin). The vessel was covered with a glass slide to avoid evaporation of the liquid and to prevent contaminations of the cell monolayer.

2.2. Gas exposure

The gas exposure was done like described before [3]. Briefly, the cell covered sensor chips were placed inside the biomodules of the analyzing system and used in an alternating stop/go modus (3 min/3 min) in liquid medium. Immediately before the gas exposure, pumps were stopped to prevent further transport of liquid medium to the cells. The liquid medium was sucked off completely from the cell monolayer on the chip to insure its full contact with the gas phase. A T-shaped device (see Figure 1a) was plugged on the culture vessel of the sensor chip which leads the test air over the cells. The test air was basically composed of 80% N_2 and 20% O_2 carrier gas and humidified to 70% r.h. at room temperature. After exposure duration of 10 minutes, the cells are re-immersed in liquid medium. The perfusion head is placed inside the chip vessel and the stop/go modus keeps on pumping fresh medium over the cells.

2.3. MIR-measurements

MIR-measurements were done using a Bruker (Ettlingen, Germany) IFS 66v FT-IR spectrometer. Metabolic chips containing a monolayer of V79 cells were used for the spectroscopic investigations to prove the quality and the reproducibility of the used medium draining method. The chips were placed inside the IR-spectrometer and the light beam was focused to the centre of the cell monolayer. Three types of sensors were compared: (i) sensor chips with a V79 cell monolayer covered with 200 μL nutrient medium; (ii) sensor chips with a V79 cell monolayer where the medium was totally removed and dried in a nitrogen flow for 100 minutes; (iii) sensor chips with a V79 cell monolayer immediately after the medium was drained of.
The difference in the signal pattern of cell monolayer covered with aqueous nutrition media and a cell monolayer extensively dried in a nitrogen flow for 100 minutes were compared with the signals of a cell monolayer directly after the draining of the nutrient media with the peristaltic pump. Mid-infrared spectroscopy is a highly sensitive method for the detection of aqueous solutions as it generates prominent signals in the area of the H-O vibration around 1600 cm^{-1}.



(a) (b)

Fig. 1. (a) Adapted plug-on device for the direct gas exposure of living cells on a sensor chip. (b) Real-time monitoring of the adhesion behavior of A549 cells to investigate influence of extracellular matrix proteins on the signal stability of the cell-substrate contact.

3. Results and Discussion

3.1. Results of cell adhesion measurements

Impedance measurements are widespread for the analysis of cell adhesion towards a specific substrate [4]. As the cells spread, they alter the effective area available for current flow, causing a significant decrease in the capacitance as shown in Figure 1b. In the case of A549 cells on a sensor chip with protein coating, surprisingly the signal stability is lower and therefore, the capacitance values are higher even after several hours of settlement (see Fig. 1b).

3.2. Results of gas exposure

RPMI2650 cells react towards ammonia diluted in synthetic air with a stable decrease of the acidification rate of about 10% for 40 ppm NH_3 and 30% for 66 ppm NH_3 (see Figure 2a). Possibly ammonia, a widely known cell toxin, reacts with α-ketoglutarate, a component of the citric acid cycle, and thereby inhibits the ATP production.

3.3. Results of MIR-measurements

MIR-spectroscopy is an analytical method whose signals can be easily interfered by humidity. Therefore we used it to control the residue of aqueous medium on top of the cells which might inhibit the direct contact of the probe gas with the cell monolayer. Medium covered cells showed a strong water signal in the range of the H-O vibration (1600 cm^{-1}; see black curve in Figure 2b). The water signal is almost completely disappeared after draining off the media and additional treatment with a nitrogen flow for 100 minutes (see green curve in Figure 2b).

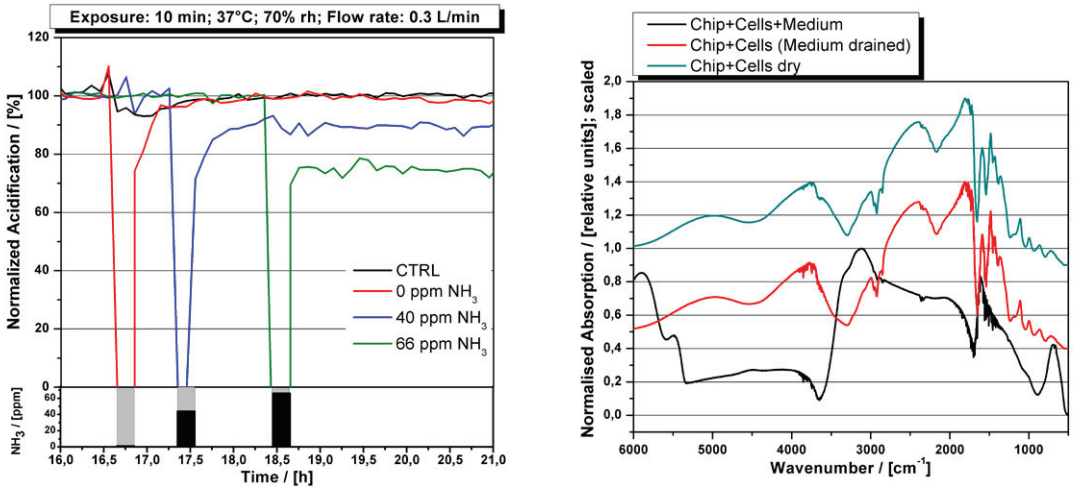


Fig. 2. (a) Changes of normalized acidification rates due to the exposure of RPMI2650 cells to different concentrations of ammonia gas diluted in synthetic air for 10 minutes. Grey bars indicate exposure to the carrier gas. The measurements were done in parallel. (b) MIR-spectra of V79 cells on a Bionas sensor chip. Red curve shows the sufficiency of the draining method as the characteristics of the curve representing the completely dried cells (green curve; 100 min N_2) and the medium covered cells (black curve; 200 µL medium) are the same.

4. Conclusion

Multiparametric cell-based gas biosensors seem to be suitable tools for the detection of soluble and insoluble toxic gases in air. The presence of toxic gas leads to the impairment of the living cells expressed by a sustainable reduction of acidification- and respiration rates [3]. Mechanical stability and tolerance towards shear forces produced by the air flow are increased by using extracellular matrix proteins as coatings for the sensor chip surface. MIR-spectroscopy is a sensitive method for the measurement of residual water and therefore can be used as a quality control of the draining method. This sensor system may be used in technology fields like environmental monitoring or biomedical applications.

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